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TITLE: Activation and Protection of Dendritic Cells in the Prostate Cancer Environment

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13. SUPPLEMENTARY NOTES

14. ABSTRACT Third annual report for this award. Experiments were conducted as was scheduled in the Statement of Work. So far studies have demonstrated for the first time the presence of endothelin receptors on murine DC, and the fact of endothelin-1 production by murine DC upon stimulation with TNF• and lipopolysaccharide (LPS). The modification of the endothelin axis on DC changed its resistance against prostate cancer induced apoptosis – the blockade of ETA receptors resulted in the increased apoptotic rate, while the blockade of the ETB receptors lead to the increased survival of DC in the prostate cancer environment. Based on these data, in vivo experiments were carried out, in which mice with prostate cancer (RM1 cells) were treated with intratumoral injection of modified DC (stimulated DC, with ETB receptor blocked). This treatment resulted in reduction of prostate cancer growth in mice in the experimental group, in comparison to untreated control mice. Studies are under way to elicit the mechanisms of endothelin axis action on DC, as well as the underlying mechanisms of interaction between dendritic cells and prostate cancer cells.

15. SUBJECT TERMS
Prostate cancer, Dendritic cells, Endothelin receptors, Immunotherapy

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Introduction:

This study is being conducted for the (i) characterization of the prostate cancer and dendritic cells (DC) interaction; (ii) defining the role of endothelin axis in the maturation of DC, (iii) elucidating the role of endothelin axis in the prostate cancer-DC interaction, and (iv) modification of dendritic cells to be used in the treatment of prostate cancer. Mouse model will be used. This is the report for the first 3 years of the award. Experiments are progressing according to the plan so far. Experiments reported during the prior annual reports will be discussed as well during this submission.

Report:

First task was to proceed with the characterization of role of endothelin axis in DC. For this purpose, DC were grown from C57BL/6 mice bone marrow, as was described earlier [1]. Briefly, bone marrow cells were first depleted of RBC with lysing buffer for 2–3 min. The single-cell suspensions then was incubated with a cocktail of Abs (αCD4, αCD8a, and B220) for 1 h at 4°C, followed by incubation with rabbit complement for 30 min at 37°C to deplete cells expressing lymphocyte Ags B220, CD4, and CD8. Cells were then incubated overnight (37°C, 5% CO_2) in six-well plates (Falcon, Franklin Lakes, NJ) at a concentration of 10^6 cells/ml in complete medium, consisting of RPMI 1640, 2 mM L-glutamine, 50 μg/ml gentamicin sulfate, 10 mM HEPES, 10% FBS, 0.1 mM nonessential amino acids, and 1 mM sodium pyruvate (Life Technologies). The nonadherent cells were collected by gentle pipetting and resuspended at a concentration of 2.5 x 10^5 cells/ml in complete medium supplemented with 1000 U/ml recombinant murine GM-CSF and recombinant murine IL-4 (R&D system). Cells were cultured in six-well plates (4 ml/well) for 7 days at 37°C in 5% CO_2. Nonadherent DC are collected by gentle pipetting, counted, characterized as described previously [2], and used for further studies.

For the characterization of the general impact of endothelin receptors, dendritic cells were stimulated with TNFα and lipopolysaccharide (LPS, Sigma-Aldrich) for the endothelin production and the expression of endothelin receptors, since our preliminary data indicated increased expression of endothelin receptors upon stimulation in mice (unpublished data). We have previously demonstrated as well increased production of endothelin-1 (ET-1) by human DC, and increased expression of endothelin receptors [3].

For the characterization of ET-1 production, DC were cultured as described above, and stimulated with TNFα (10ng/ml, added on Day 5) or LPS (200ng/ml, added on Day 5) for 48 hours. After that, cell-free supernatants were collected, and ET-1 concentrations were measured using ET-1 enzyme-linked immunosorbent assay (ELISA) system (R&D systems, Minneapolis, MN); in every assay, samples and standards were run in duplicates and read at 450-nm wavelength on a microplate reader. ET-1 concentrations were normalized based on cell counts and determined by computer software–generated interpolation from the standard curve.

As it can be seen on Fig.1, control DC (unstimulated) produced 22.67±2.34 pg/ml/mln cells. TNFα stimulated cells produced 67.35±6.92 pg/ml/mln cells (The difference was statistically significant, $P<0.001$), and LPS-stimulated celles produced 84.24±3.84 pg/ml/mln cells (The difference was statistically significant – $P<0.001$, in

comparison to control. There was no statistically significant difference in ET-1 production levels among cells stimulated with TNFα or LPS).

Next, we evaluated the expression of the endothelin receptors upon stimulation. DC were cultured as usual, and stimulated with TNFα and LPS as described above. Cells were collected on day 7, air dried and fixed in methanol at -20°C for 20 min. After the slides are washed with PBS, they are blocked in 10% normal goat serum (1 hr in room temperature) and incubated with antibodies against ET_A or ET_B receptors (1:50 dilution, Alomoni Labs, Israel) at 4° C overnight. Following PBS wash (5 min x 2), the cells were incubated with fluorescent secondary antibody at room temperature for 1 hr. Immunostaining of ET receptors was examined with a Nikon fluorescent microscope. The results are presented on Figure 2. As it can be seen, the the stimulation of DC indreased the expression of endothelin receptors.

Changes in phenotype has been evaluated as well. Murine DC has been stimulated with TNFα on day 5 for 48 hours. Stimulated DC were treated with endothelin receptor inhibitors BQ-123 (Selective ET_A receptor inhibitor, American Peptide Company), at a final concentration of 10^{-6} *M*, for the last 48 hours, and with BQ-788 (Selective ET_B receptor inhibitor, American Peptide Company), at a final concentration of 10^{-6} *M*, for the last 48 hours as well. After that, cells were collected, washed, counted and stained for flow cytometry. We have evaluated cells for the expression of CD40, CD80, CD86, MHC class II antigen, and CD205. Briefly, stimulation with TNFα resulted in the increased expression of these costimulatory molecules (as expected). The blockade of ET_A receptor with BQ-123 induced in general decreased expression of the costimulatory molecules, which was especially significant for CD40 and CD205 (difference was statistically significant by chi-square test, $P<0.001$). On the other hand, the blockade of ET_B receptor with BQ-788 resulted in no change or increased expression of costimulatory molecules (Fig.3).

For the further characterization of the endothelin axis on dendritic cells, we proceeded with the mixed leukocyte reaction (MLR). Briefly, allogeneic T cells were generated from balb mice spleens using murine T cell enrichment columns (R&D Systems, Minneapolis, MN). Isolated T cells were placed in the round-bottom 96-well plates, $3x10^5$ per well, and DC were added with decreasing concentrations. After 72 hours of incubation at 37°C, ^{3}H-TdR (New England Nuclear Co., Boston, MA) was added to the DC/T cell mixture, 1μCi per well. T cell proliferation was measured by the ^{3}H-TdR uptake in 16 hours. Cells were harvested onto glass fiber filter paper with a semi-automated microharvester, and ^{3}H-TdR incorporation was determined by liquid scintillation spectroscopy. There were four experimentl groups: 1) DC prepared as usual; 2) DC treated with TNFα during the last 48 hours; 3) DC treated with TNFα and BQ-123 for the last 48 hours, and 4) DC treated with TNFα and BQ-788 for the last 48 hours. Preliminary results of the experiment are presented in the Figure 4. As it can be seen, addition of TNFα resulted in increased ability of the DC to stimulate T cells. While the addition of the BQ-788 didn't produce significant changes in these preliminary experiments, addition of BQ-123 resulted in decreased ability of DC to stimulate T cells, in comparison to DCtreated with TNFα alone. These results lead us to speculate that the stimulation of ET_A receptors may lead to the activation of DC, and that their blockade might abolish or lessen immune response.

During the next set of experiments, the influence of prostate cancer cells on DC was evaluated. DC were grown as described above. On days 5 and 6, 1 ml of RM-1

(murine prostate cancer cells) cells supernatant was added to each well (in the 6-well plates). Control DC received media only (1 ml per well on days 5 and 6). On day 7, cells were collected, and flow cytometry was performed for the expression of costimulatory molecules. Results are presented on Figure 5. Addition of tumor cells supernatant resulted in the 10% drop in the expression on CD80 marker, the expression of CD205 was reduced significantly as well.

Next, we evaluated the influence of endothelin receptors on DC survival in the prostate cancer environment. As we have already demonstrated previously [3], blockade of ETA receptors worsened DC survival, while the blockade of ETB receptors improved DC resistance against apoptotic stimuli. Now, in mice model, we evaluated the influence endothelin axis modification on DC survival in the prostate cancer environment. We performed the incubation of murine DC with RM-1 cells (murine prostate cancer cells), which resulted in DC apoptosis (apoptotic rate 30.96±3.9%). Pretreatment of DC with TNFα lowered apoptotic rate to 25.37±2.7%. Blockade of ET_A receptors with BQ123 increased prostate cancer-induced DC apoptosis to 45.45±4.6%. Blockade of ET_B receptors with BQ788 improved DC resistance to prostate cancer-induced apoptosis and dropped apoptosis rate to 16.90±3.3% (Fig. 6).

In vivo experiments were performed as well. Tumors were induced by subcutaneous injection of 25,000 RM-1 murine prostate cancer cells into groups (n=5) of the C57BL/6 mice. When tumors became palpable (day 6), treatment was initiated with injection of $1.5x10^6$ bone marrow derived DC into the tumor. Group 1 received Hank's solution (control); Group 2 – unmodified DC; Group 3 –DC treated with TNFα during the last 48 hours; Group 4 - DC treated with TNF-α and ET_B receptor antagonist BQ-788 during the last 48 hours (our previous studies have shown the increased expression of endothelin receptors after the stimulation of DCs with TNFα, and improved DC survival with the blockade of ET_B receptors). Two further injections were performed on days 9 and 12. Tumor size was assessed starting from day 14 until animal sacrifice. By day 24, mean tumor size reached 1796±166 mm^3 in the Group 1 (control), 1556±186 mm^3 in the Group2, 1508+166 mm^3 in the group 3, and 397±186 mm^3 in Group 4 ($P<0.001$ versus control). Difference in mean tumor size became significant starting from day 20 (figures 7 and 8).

One gene array experiment was performed, to assess the influence of prostate cancer cells on DC. Briefly, 7-day-old cultured DC wetre harvested and co-incubated with the murine prostate cancer cell line RM-1 in six-well plates. DC and tumor cells were separated using membrane inserts with 0.4-μm pore size, which exclude direct cell-to-cell contact, but allow free exchange of soluble factors. Specifically, 5×10^5 DC will be placed in six-well plates in 3 ml of medium. One million prostate cancer cells resuspended in 2 ml of medium were placed into the inserts on the top of each well. As controls, DC were coincubated with murine splenocytes. DC were harvested 48 h later, washed, RNA was extracted using RNA extraction minikit, and used for gene arrays. We used mouse 22K Oligo Arrays (Center for Applied Genomics) which is composed of fifteen-thousand 70 mer oligonuclotides corresponding to specific mouse transcripts. The oligonucletides were spotted onto poly-lysine-coated glass microscope slides by using a Gene Machines Omnigrid 100 arrayer (Genomic Solutions, Ann Arbor, Mich.) and SMP3 pins (Telechem, Sunnyvale, Calif.). RNA labeling and hybridization was performed using the 3DNA Array detection Array 350 Kit (Genisphere Inc.) according to the manufacturer's instructions.

We used "comparison design" for this experiment, were RNA's were compared to each other directly, without standard. Preliminary analyze of data demonstrated so far decreased expression of receptors for IL-12 and interferon gamma in DC incubated with RM-1 cells. More experiments with "reference settings" are scheduled.

Key research Accomplishments:

- Production of ET-1 by murine DC has been documented first time, as well as the presence of endothelin receptors on murine DC.

- The influence of endothelin receptor inhibitors on DC phenotype was demonstrated. Functional experiments (MLR) demonstrated the possible involvement of the ET_A receptors in the activation of DC, driving them towards TH1 response. It seems that ET_B receptor stimulation might drive DC toward tolerance, with decreased expression of co-stimulatory molecules. Further studies are needed to clarify the exact role of these receptors in DC biology. In vivo studies are scheduled.

- Treatment of DC with prostate cancer cells supernatants induced decreased expression of some co-stimulatory molecules.

- We have demonstrated for the first time that the modification of endothelin axis on dendritic cells may result in increased resistance and improved survival against prostate cancer cells.

- Treatment of murine prostate cancer by intratumoral injection of the modified dendritic cells resulted in the reduction of the tumor growth. These data may provide basis for the development of clinical trials protocol.

Reportable outcome:
Research data have been presented at the Annual meetings of the American Association for Cancer Research (AACR), and American Urological Association (AUA), as well as at the IMPaCT Meeting (September 5-8, 2007, Atlanta, GA). The Abstract of the presentation at the AUA is attached.

Conclusion:

So far experiments have demonstrated the possible role of endothelin receptor inhibitors in the function of DC, which can be useful in the treatment of different diseases, ranging from cancer to transplantation. Our in vivo experiments showed the possible role of endothelin receptors modification on DC in the treatment of prostate cancer in mice. More experiments are underway, and clinical trials protocol is being planned for patients with advanced prostate cancer, using modified autologous DC.

Research data have been submitted and accepted for the presentation at the AUA 2007 and AACR 2007 annual Meetings. Final copy of the abstract is attached.

References:

1. Pirtskhalaishvili G, Shurin GV, Gambotto A, Esche C, Wahl M, Yurkovetsky ZR, Robbins PD, Shurin MR. Transduction of dendritic cells with Bcl-xL increases their resistance to prostate cancer-induced apoptosis and antitumor effect in mice. Journal of Immunology. 2000;165:1956-1964

2. Shurin MR, Pandharipande PP, Zorina TD, Haluszczak C, Subbotin VM, Hunter O, Brumfield A, Storkus WJ, Maraskovsky E, Lotze MT. FLT3 ligand induces the generation of functionally active dendritic cells in mice. Cell Immunol. 1997;179:174-184

3. Guruli G, Pflug BR, Pecher S, Makarenkova V, Shurin MR, Nelson JB. Function and survival of dendritic cells depend on endothelin-1 and endothelin receptor autocrine loops. Blood. 2004;104:2107-2115

Appendices:

Figure 1.
Assesment of ET-1 production DC. Dendritic cells were cultured as usual, and stimulated during the last 48 hours either by TNFα or LPS. Supernatants were collected and ET-1 level was measured using ELISA test. Control DC (unstimulated) produced 22.67±2.34 pg/ml/mln cells. TNFα stimulated cells produced 67.35±6.92 pg/ml/mln cells (The difference was statistically significant, P<0.001), and LPS-stimulated celles produced 84.24±3.84 pg/ml/mln cells (The difference was statistically significant – P<0.001, in comparison to control. There was no statistically significant difference in ET-1 production levels among cells stimulated with TNFα or LPS).

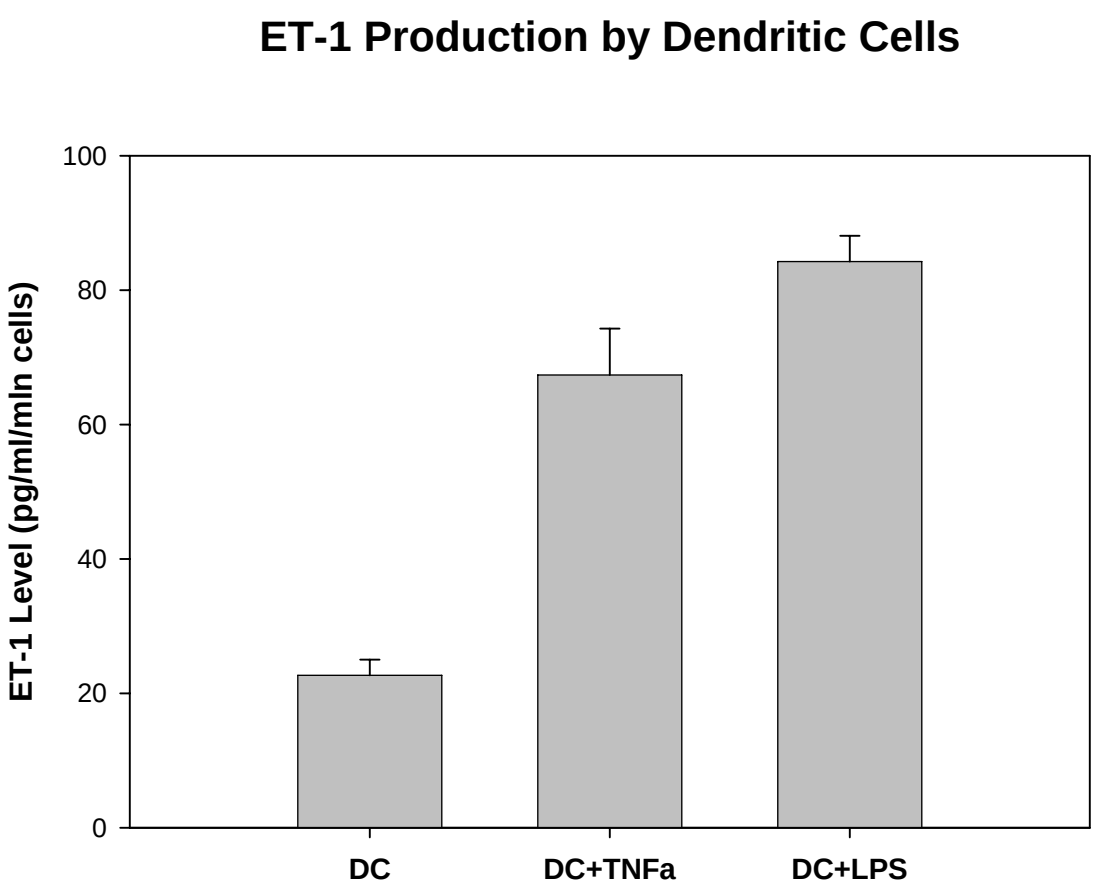


Figure 2.
Increased expression of endothelin receptors on murine dendritic cells after stimulation.
A and B, stained with ET_A antibodies,
C and D, stained with ET_B antibodies.
A and C, Unsimulated (control) DC.
B and D, Stimulated (with LPS, as described in the text) DC.

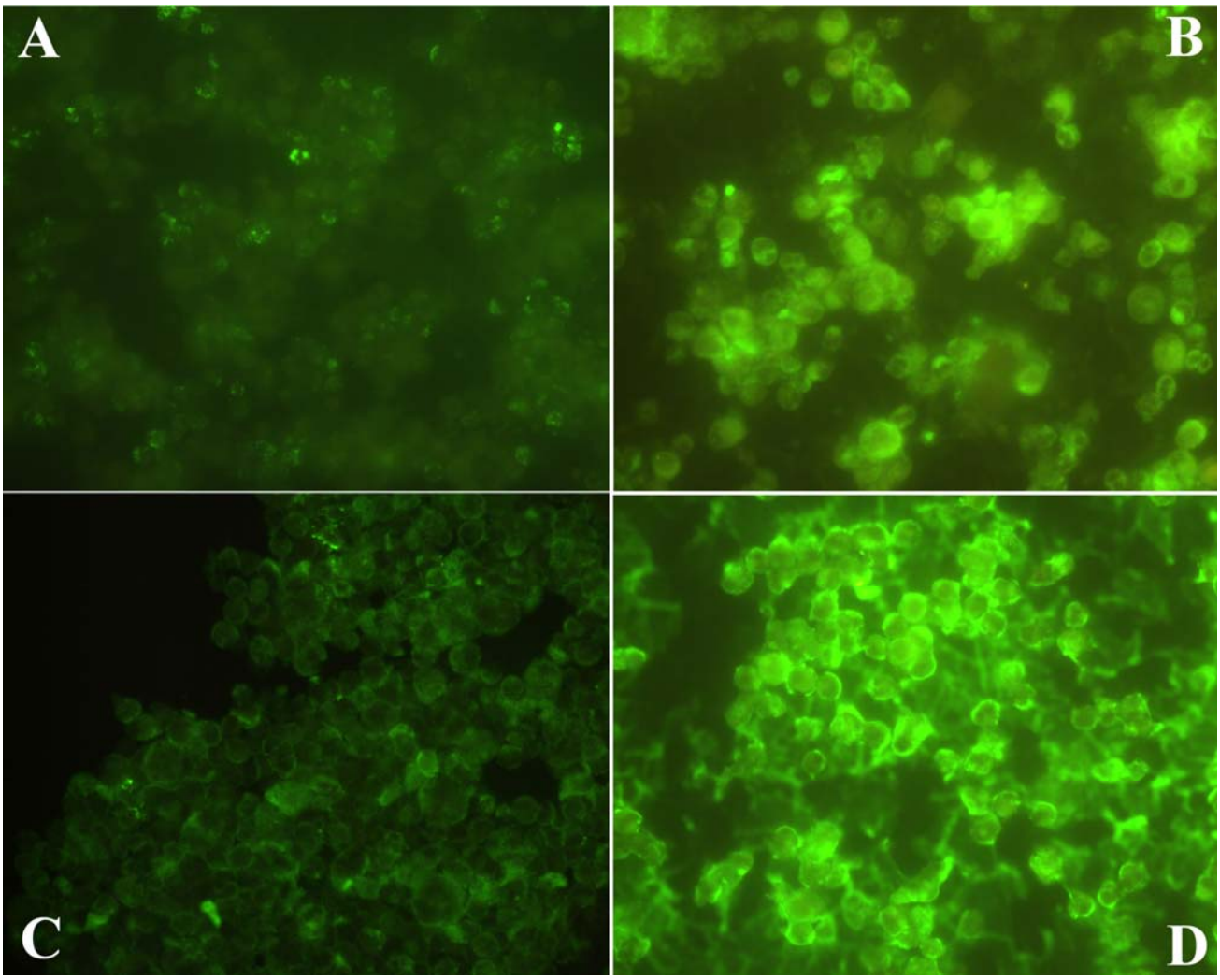


Figure 3.
Phenotyping of murine dendritic cells (DC) stimulated with TNFα and treated either with ET_A receptor inhibitor (BQ-123) or ET_B receptor inhibitor (BQ-788). Blockade of ET_A receptors resulted in the decrease of the costimulatory molecule expression, while ET_B receptor blockade was accompanied by mild increase in the expression of costimulatory molecules.

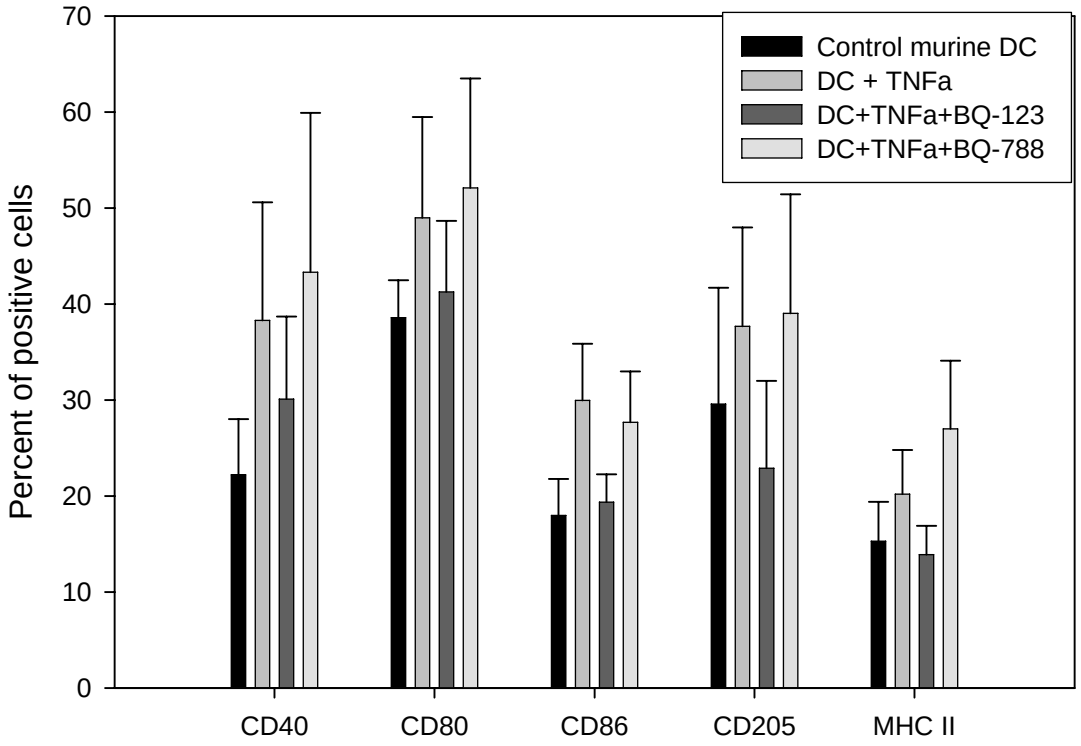


Figure 4.
The influence of the endothelin receptor inhibitors on DC is shown. Briefly, DCs were treated either with TNFα, with TNFα+BQ123 (ET_A receptor inhibitor), and with TNFα+BQ-788 (ET_B receptor inhibitor). Untreated DC provided control. Modified DC were used to stimulate T cells in the mixed leukocyte reaction.

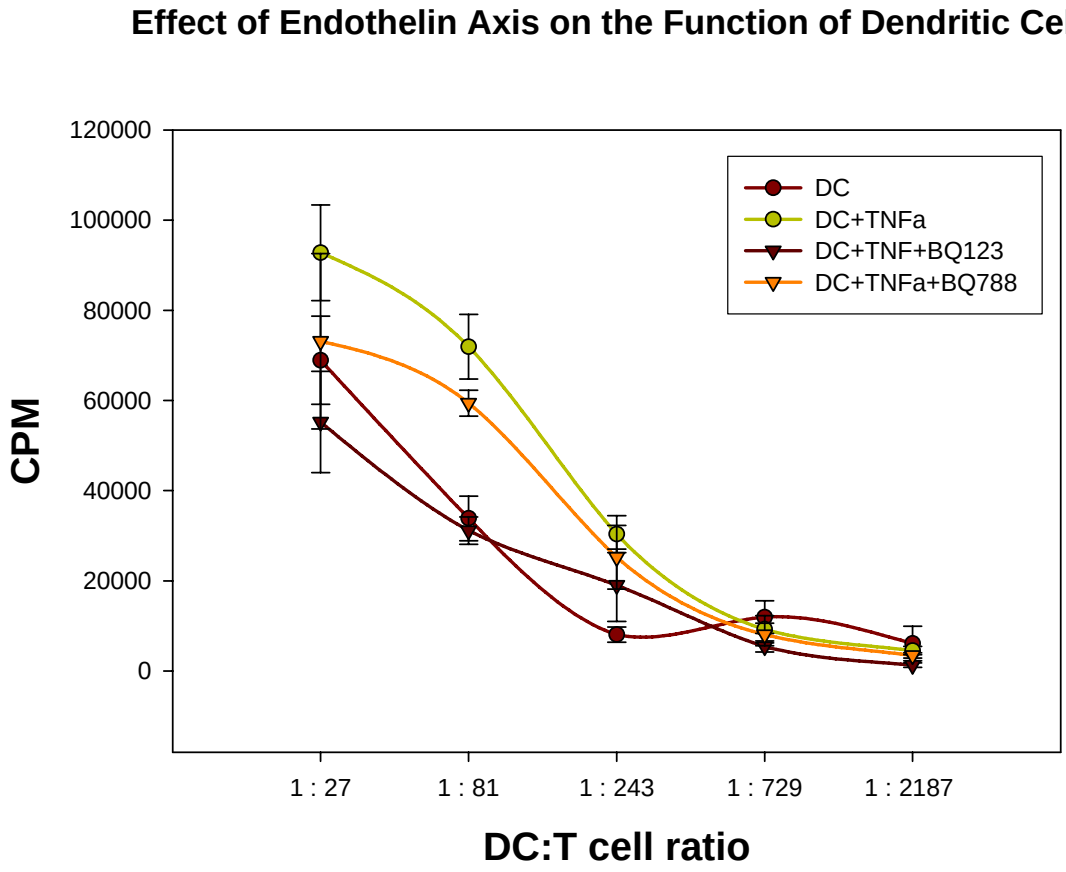


Figure 5.
Dendritic cells were grown in the culture as usual. On days 5 and 6, 1 ml of RM-1 cells (prostate cancer cells) supernatant was added to the culture. Control DC received 1 ml of media. Cells were collected on day 7 and flow cytometry was performed for the expression of co-stimulatory molecules.

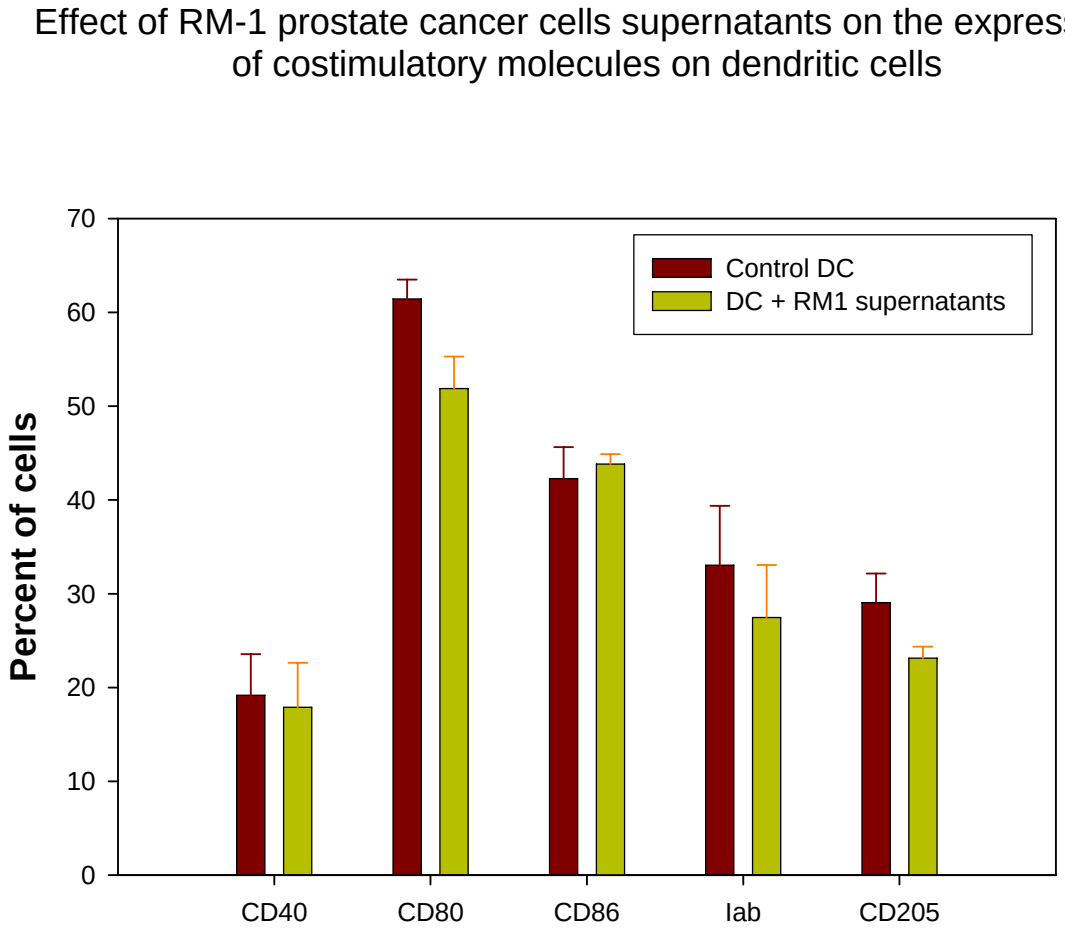


Figure 6.
Protection of dendritic cells from prostate cancer-induced apoptosis. Incubation of murine DC with RM-1 cells (murine prostate cancer cells) resulted in DC apoptosis (apoptotic rate 30.96±3.9%). Pretreatment of DC with TNFa lowered apoptotic rate to 25.37±2.7%. Blockade of ETA receptors with BQ123 increased prostate cancer-induced DC apoptosis to 45.45±4.6%. Blockade of ETB receptors with BQ788 improved DC resistance to prostate cancer-induced apoptosis and dropped apoptosis rate to 16.90±3.3%. Data of representative experiments are presented.
A – DC + RM-1
B – DC + TNFα + RM-1
C – DC + TNFα + BQ123 + RM-1
D – DC + TNFα + BQ788 + RM-1

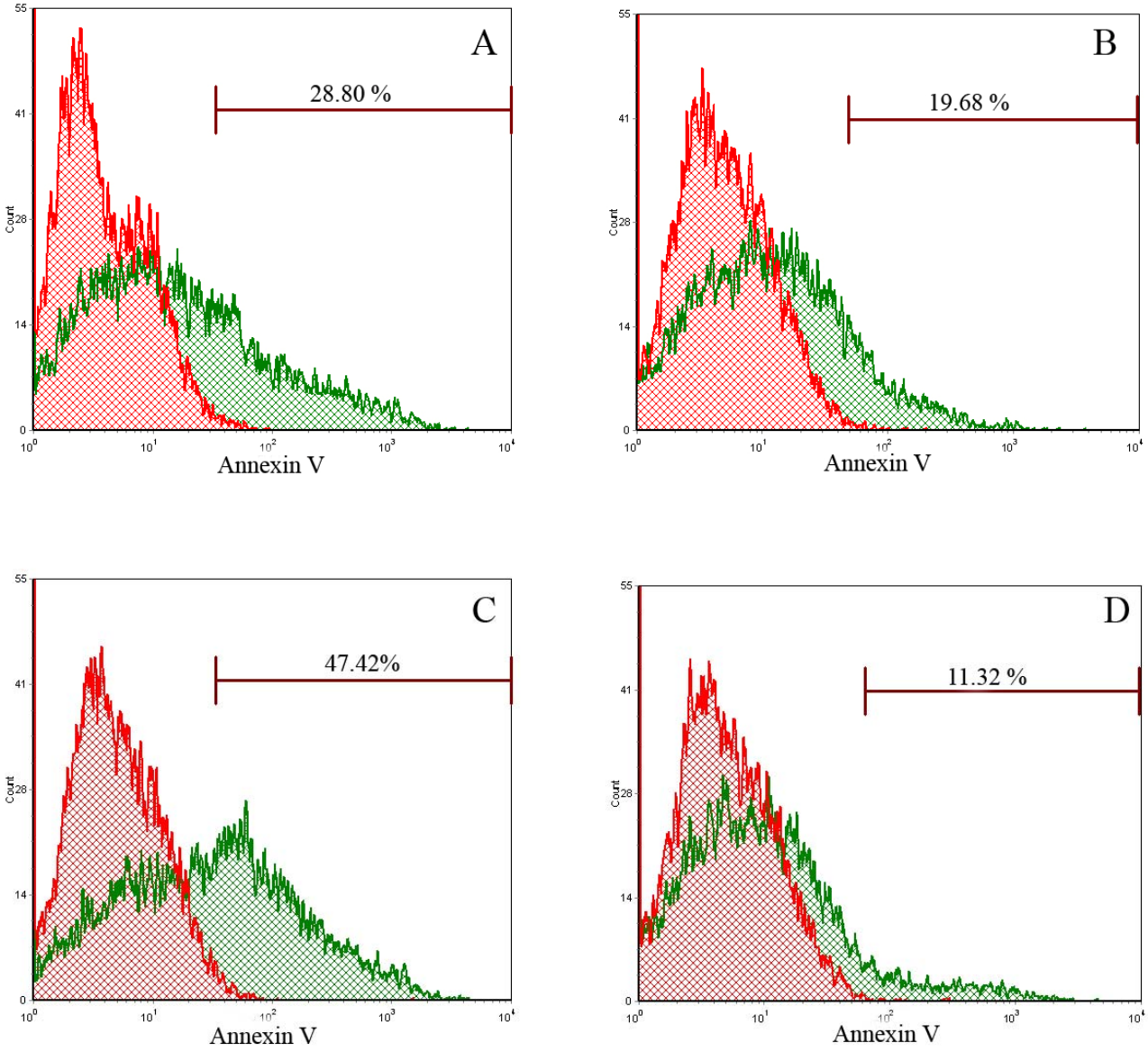


Figure 7.
Mice were injected with RM-1 murine prostate cancer cells. Treatment was started on day 6 by intratumoral injection of the dendritic cells on day 5. Group 1 (control) mice were injected with the vehicle (HBSS), Group 2 - with unmodified DC, Group 3 - with DC tretaed by TNFα, and group 4 - treated with TNFα and BQ-788 (ET_B receptor inhibitor). Tumor size was measured twice a week.

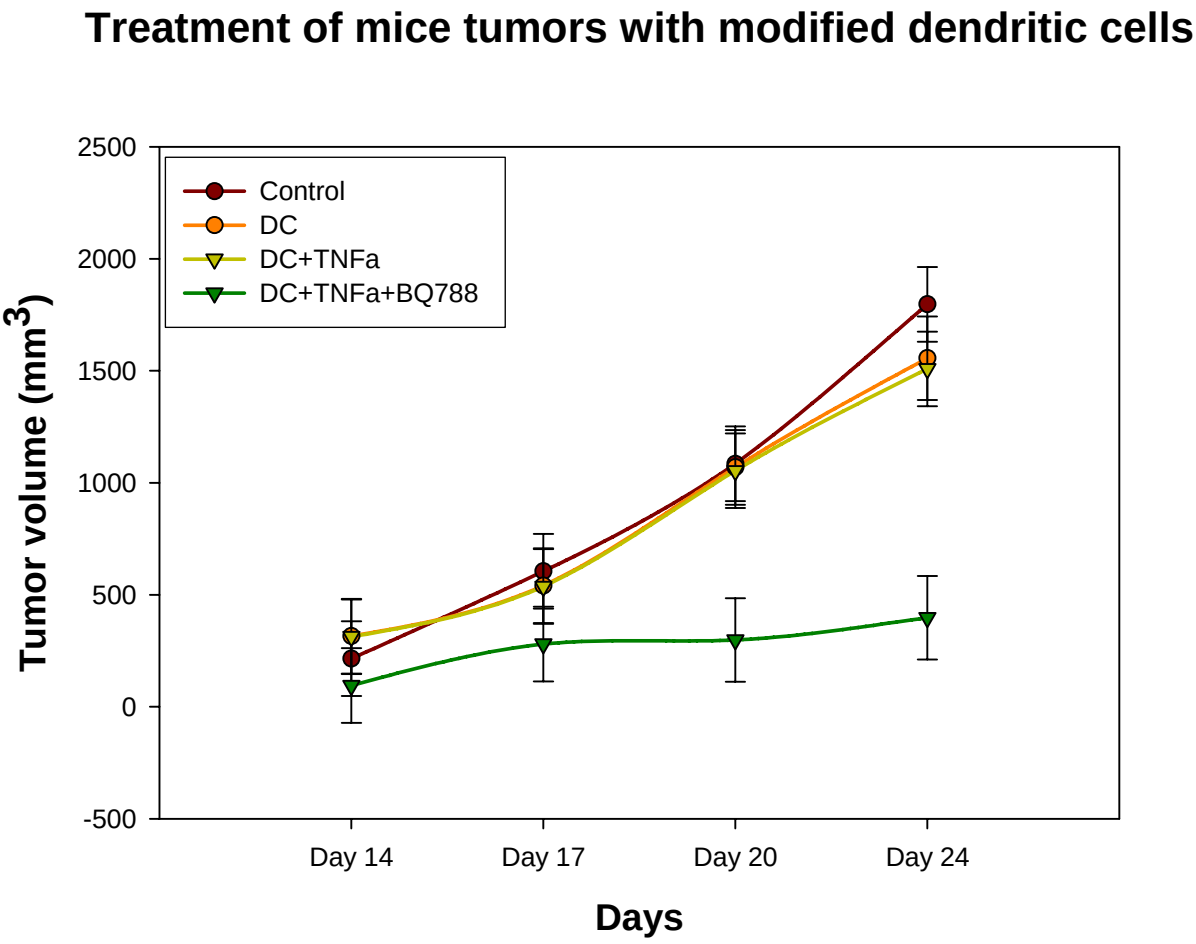
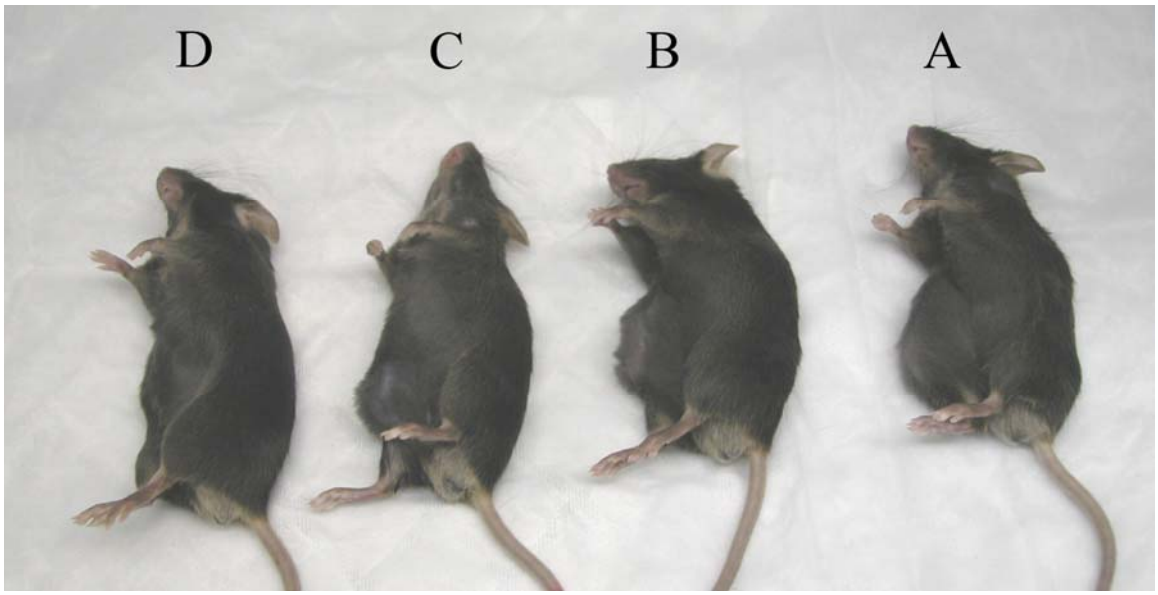


Figure 8.
Mice were injected with RM-1 murine prostate cancer cells. Treatment was started on day 6 by intratumoral injection of the dendritic cells on day 5. Group 1 (control) mice were injected with the vehicle (HBSS), Group 2 - with unmodified DC, Group 3 - with DC tretaed by TNFα, and group 4 - treated with TNFα and BQ-788 (ET_B receptor inhibitor). Tumor size was measured twice a week. Pictures of representative mice are presented. A – treated with HBSS, B – treated with DC, C – treated with DC+TNFα, D – treated with DC+TNFα+BQ-788.



Presented at the Annual Meeting of the AUA (May 2007, Anaheim, CA).

Prostate Cancer: Basic Research (III)

Moderated Poster
Sunday, May 20, 2007 3:30 PM - 5:30 PM

#531
USE OF MODIFIED DENDRITIC CELLS FOR THE TREATMENT OF PROSTATE CANCER IN MICE

Georgi Guruli, Renee Kancelarich, Peter Hinds, Sean Taheri, Mark L Jordan. Newark ,NJ.*

Introduction and Objective: Immunotherapy via dendritic cells (DC) has shown promise as a novel approach to hormone-refractory prostate cancer. DC are the major antigenpresenting cells and regulators of the immune response. We have previously demonstrated that prostate cancer induces DC apoptosis, which could be one mechanism of tumor escape from immune surveillance. We also demonstrated the presence of endothelin receptors on DC, and that blockade of endothelin B (ET_B) receptors decreases DC apoptosis, while it may enhance their antigen presenting ability. The purpose of the current study was to determine whether ET_B blockade also enhances DC antitumor activity in a murine prostate cancer model. Methods: Tumors were induced by subcutaneous injection of 25,000 RM-1 murine prostate cancer cells into groups (n=5) of the C57BL/6 mice. When tumors became palpable (day 6), treatment was initiated with injection of $1.5x10^6$ bone marrow derived DC into the tumor. DC were prepared as usual in the complete media in the presence of the GM-CSF and IL-4 for 7 days. Group 1 received Hank's solution (control); Group 2 - unmodified DC; Group 3 -DC treated with TNF-α during the last 48 hours; Group 4 - DC treated with TNF-α and ET_B receptor antagonist BQ-788 during the last 48 hours (our previous studies have shown the increased expression of endothelin receptors after the stimulation of DC with TNF-α). Two further injections were performed on days 9 and 12. Tumor size was assessed starting from day 14 until animal sacrifice. Results: By day 24, mean tumor size reached 1796 ± 166 mm^3 in the Group 1 (control), 1556 ± 186 mm^3 in the Group 2, $1508+166$ mm^3 in the group 3, and 397 ± 186 mm^3 in Group 4 ($P<0.001$ versus control). Difference in mean tumor size became significant starting from day 20. Conclusions: We hypothesize that ET_B receptor blockade protects DC from apoptosis and may increase their antigenpresenting capability. Our data suggest that ET_B receptor blockade also promotes DC antitumor activity *in vivo*. We plan to extend these studies into human clinical trials, using autologous dendritic cells.
Source of Funding: Department of Defence - Physician Research Training Grant (GG); Veterans Administration merit Review Award (MLJ); UMDNJ Foundation (MLJ); New jersey Medical School Dean's Research Fund (MLJ)

Curriculum Vitae

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Email: gurulige@umdnj.edu,

1. Education

   a. Undergraduate N/A
   b. Graduate
      Tbilisi State Medical Institute, Tbilisi, Georgia (former USSR)
      Degree: **M.D.,** Date Awarded: June 26, 1983

2. Post Doctoral Training

   a. Internships and Residencies
      i. Clinical ordinatura (residency):
         Location: Georgian Oncological Research Center, Tbilisi, Georgia
         Discipline: Surgical Oncology
         Inclusive Dates: 09/1983 – 09/1985.
      ii. Internship (PGY-I):
         Location: University of Pittsburgh Medical Center, Pittsburgh, PA
         Discipline: Surgery
         Inclusive dates: 07/1995 – 06/1996.
      iii. Residency (PGY-II):
         Location: University of Pittsburgh Medical Center, Pittsburgh, PA
         Discipline: Surgery
         Inclusive dates: 07/1996 – 06/1997.
      iv. Residency (PGY-III – PGY-VI):

Location: University of Pittsburgh Medical Center, Pittsburgh, PA
Discipline: Urology
Inclusive dates: 07/1997 – 06/2001.

b. Research Fellowships

i. Research Fellow:
National Oncological Research Center, Moscow, USSR
Discipline: Urologic Oncology
Inclusive Dates: 09/1987 – 12/1990.
Ph.D. Degree awarded on 12/08/1990.

ii. Research Fellow:
University of Pittsburgh School of Medicine, Pittsburgh, PA
Discipline: Urologic Oncology
Inclusive Dates: 07/2001 – 11/2003.

3. Licensure (state, specialty, issue date, expiration date)

a. Commonwealth of Pennsylvania – Medical Physician and Surgeon,
Initial License Date: 03/05/1999.
b. State of New Jersey – Medical Doctor,
Initial license date: 09/08/2003.

4. Narcotics Certification (state, dates)

CDS registration, Date issued – 10/16/2003.
DEA registration, Date issued – 03/15/2002.

5. University Appointments:

Department: Surgery, Division of Urology
UMDNJ – New Jersey Medical School
Title: Assistant Professor
Inclusive dates: 12/2003 – present.

6. Hospital Appointments

Department of Surgery, Division of Urology
Hospital Name: Georgian Oncological Research Center
Title: Staff physician
Inclusive dates: 1985-1995

Department of Surgery, Division of Urology
University Hospital, Newark
Title: Attending physician
Inclusive dates: 12/2003 – present.

7. Awards and Honors

1977	Gold Medal (Highest Honors), High School #1, Tbilisi, Georgia
1983	Highest Honors ("Red Diploma"), Tbilisi State Medical Institute, Georgia
1998	Second Prize, Clinical Section, Pittsburgh Urological Society Meeting, Pittsburgh, Pennsylvania
1999	Pfizer Scholars in Urology Award.
1999	Best Basic Science Paper Award, 51st Annual Meeting of Northeastern Section, AUA. Bermuda, UK.
1999	First Prize, Basic Research Section, Pittsburgh Urological Society Meeting, Pittsburgh, Pennsylvania.
2000	Resident Prize Essay Award, 52nd Annual Meeting of Northeastern Section, AUA. Pittsburgh, USA.
2002	Sylvia Sorkin Greenfield Award, for the best paper published in *Medical Physics.*
2004	AUA travel Award to attend NIDDK Clinical Research Meeting

8. Board Certification – February 2006 – By the **American Board of Urology**.

9. Principal Clinical and Hospital Service Responsibilities:

Hospital Name: Georgian Oncological Research Center, Tbilisi, Georgia
Department or Service: Urology
Responsibilities – Admission of patients in the hospital, preoperative evaluation and designing of treatment plan, administration of treatment (surgical or medical), postoperative care in the hospital, analyzing treatment outcomes, designing new treatment methods and schemas.
Inclusive Dates: 1985 – 1995.

Hospital Name: University Hospital, Newark, NJ
Department or Service: Surgery (Urology)
Responsibilities – Admission of patients in the hospital, evaluation and elaboration of treatment plan, administration of treatment, post-treatment follow-up, analyzing treatment outcomes, designing and participating in clinical trials.
Inclusive Dates: 12/2003 – present.

10. Ad Hoc Reviewer:
International Journal of Cancer
American Cancer Society
Cytokine
Medical Science Monitor
Grant reviewer for NIH

11. Memberships, Offices and Committee Assignments in professional Societies

i. European Association of Urology
Active Member
1996 – 2000.
ii. American Urological Association
Candidate Member
1997 – 2001
Associate Member
2002 – 2006
Active Member – since 2006
iii. American Association for Cancer Research
Associate Member
Dates: 1999 – 2004.
Active Member – since 2005.

12. Major Research Interests:
b. Prostate cancer:
Relationship and interaction between prostate cancer and dendritic cells (DC), the major antigen-presenting cells. To study the mechanisms of prostate cancer-induced DC suppression, and design the ways of protecting DC from apoptosis. Development of DC-based therapies of advanced prostate cancer.
c. Immunomodulation and the role of endothelin-1 (ET-1) and its receptors in the generation of immune response, in particular, the role of endothelin axis in affecting of DC function.

13. Grant History
a. Principal Investigator

i. Funding Organization: American Foundation for Urologic Disease / American Urological Association Research Scholar Program Title of Award: The Endothelin Axis: Signaling Pathways

and Maximizing Efficacy in the Treatment of Advanced Prostate Cancer.
Inclusive dates of Funding: 07/2001 – 06/2003.

ii. Funding Organization: Department of Defense, Physician Research Training Grant Title of Award: Activation and Protection of Dendritic Cells in the Prostate Cancer Environment.
Inclusive dates of Funding: 2005 – 2009.

b. Co-Investigator

i. Funding Organization: University of Pittsburgh Prostate and Urologic Cancer Center Pilot Project (Co-PI)
Title of Award: Effective Protection of Human Dendritic Cells from Prostate Cancer Induced Cell Death.
Inclusive dates of Funding: 1999-2000.

ii. Funding Organization: The Pittsburgh Foundation Program for Medical Research (Co-PI)
Title of Award: New Approach for Prostate Cancer Therapy: Dendritic Cells Protected from Tumor-Induced Death.
Inclusive dates of Funding: 1999-2002.

iii. Funding Organization: Department of Defense (DAMD17-00-1-0099 P1832735, Co-Investigator).
Title of Award: Immune Gene Therapeutic Correction and Protection of Disordered Dendritic Cells in Prostate Cancer.
Inclusive dates of Funding: 1999-2002.

14. Articles

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16. Abstracts

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22. **Guruli, G.**, Kancelarich, R., Hinds, P., Taheri, S. & Jordan, M.L. Modification of dendritic cells for the treatment of prostate cancer in mice. In *98th Annual Meeting of the American Association for Cancer Research*, Los Angeles, CA, April 14-28, 2007 (Abstract #2733), at 98th Annual Meeting of AACR.

23. **Guruli, G.**, Kancelarich, R., Hinds, P., Taheri, S. & Jordan, M.L. Use of modified dendritic cells for the treatment of prostate cancer in mice. In *102nd Annual Meeting of the American Urological Association*, Anaheim, CA, May 19-24, 2007 (Abstract #531), at 102nd Annual Meeting of AUA.

17. Patents held:

Title: **"Endothelin Axis and the Action of Dendritic Cells"**
University of Pittsburgh Case No. 00743
U.S. Patent Number: **U.S. Patent Application No. 60/513,729**
Date of Issue: pending

18. Languages spoken: Georgian, English, Russian